bio-protocol Preprint

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ATPase activity assays of SERCA reconstitutions

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An abbreviated version of this protocol was published in eLIFE in Jun 2021

Dwarf open reading frame (DWORF) is a direct activator of the sarcoplasmic reticulum calcium pump SERCA

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Detailed protocol

Calcium transport is measured as a function of ATPase activity via equivalating ADP generation as to NADH depletion through the coupling of the pyruvate kinase (PK) and lactate dehydrogenase (LDH) enzymes at 340 nm.
The assay mix (0.5mM PEP; 0.18mM NADH; 2.4mM ATP, pH 7.0; 0.5mM EGTA; 5mM $MgCl_2$; 100mM KCl, 50mM imidazole, pH 7.0) is prepared fresh for each assay. The enzymes are added last to the mix in excess at 9.6 units/mL.

For Single Cuvette Assays:
In a 1mL quartz cuvette, 750µL of assay mix and 10mM $CaCl_2$ are incubated to 25^{o}C. The $CaCl_2$ titration is achieved via the addition of 10mM $CaCl_2$ at volumes determined through Stanford free calcium concentration calculator that accounts for both the ATP, EGTA, and $MgCl_2$ concentration (http://web.stanford.edu/~cpatton/CaMgATPEGTA-TS.htm).

pCa	V of 10mM $CaCl_2$ (µL)	pCa	V of 10mM $CaCl_2$ (µL)
4.8	38.25	6.2	23.25
5.0	37.12	6.4	18.97
5.4	34.57	6.6	14.7
5.6	32.7	6.8	10.87
5.8	30.22	7.0	7.65
6.0	27.07	7.2	5.25

To initiate the reaction 1µL of 1 mg/mL calcium ionophore (in DMSO) and 20µL of the reconstituted vesicles.
Inverting 10 times before measuring NADH depletion on a Lambda 35 UV/Vis Spectrometer over a minute.

For 96-well Plate Assays:
1mM CaCl2 is made by diluting 120µL of 100mM CaCl2 into 11.88mL of assay mix.
The calcium titration curve is generated across 12 wells were both the assay mix and 1mM calcium are added at appropriate volumes to a total of 150µL per well.

pCa	V of 1mM $CaCl_2$ (µL)	V of Assay Mix (µL)
4.8	74.2	75.8
5.0	69.2	80.8
5.4	65.4	84.6
5.6	60.4	89.6
5.8	54.2	95.8
6.0	46.6	103.4
6.2	38.0	112.0
6.4	29.4	120.6
6.6	21.8	128.2
6.8	15.4	134.6
7.0	10.4	139.6
7.2	6.4	143.6

To initiate the reaction, 5.5µL of the vesicles are dispensed into each well and mixed via pipetting 25µL for 5 cycles. The absorbance is read on a BioTek Synergy 4 plate reader at 30^{o}C.
Measurement are taken every 39 seconds with mixing via shaking between measurements.
The absorbance of the solution and 96 well plate is corrected for by taking three readings at 980 nm at the beginning of the reaction.

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Fisher, M. and Young, H. (2022). ATPase activity assays of SERCA reconstitutions. Bio-protocol Preprint. bio-protocol.org/prep1819.
2. Fisher, M. E., Bovo, E., Aguayo-Ortiz, R., Cho, E. E., Pribadi, M. P., Dalton, M. P., Rathod, N., Lemieux, M. J., Espinoza-Fonseca, L. M., Robia, S. L., Zima, A. V. and Young, H. S.(2021). Dwarf open reading frame (DWORF) is a direct activator of the sarcoplasmic reticulum calcium pump SERCA. eLIFE. DOI: 10.7554/eLife.65545